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Analysis of seed protein of 29 lines of *Capsicum annuum* L. by polyacrylamide gel electrophoresis

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Abstract

Proteins and enzymes are important parameters in biochemical taxonomy. Seeds of 29 lines of *C. annuum* from different sources and places of origin were studied. The protein content was estimated and SDS polyacrylamide gel electrophoresis technique was used to study the soluble protein pattern. Each genotype was distinct from the other, but certain bands were shared by several genotypes. Average similarity was highest (80%) between accessions PBC 436 from Portugal and Round Ornamental collected from Kerala Agricultural University, suggesting that the material collected from India could have originated in Portugal. In general, paprika lines had a high seed protein content and these also clustered together. Fruit length, fruit colour, and percentage of capsaicin did not show correlations with the clustering of the accessions.

Introduction

Proteins and enzymes, characterized as primary gene products, are important parameters in biochemical taxonomy. Storage proteins separated by electrophoretic methods are thought to undergo the process of evolution with relative slowness due to their ''non-essential nature'' (Margoliash and Fitch 1968), while enzymes are thought to be extremely sensitive to selection pressures in evolution and thus to survival of the organism (McDaniel 1970). Analysis of proteins and isozymes is a tool for supplementing the evidence obtained by comparative morphology, breeding experiments and cytological analysis.

Morphometric and seed protein analysis were used to reveal differences among species and populations of *Trifolium* (Sheidai et al. 1999), characterization of *Solanum* (Menella et al. 1999) and *Ricinus communis* (Varier et al. 1999). Seed protein electrophoresis for the study of phylogenetic relationship in *Capsicum* L. was performed by Panda et al. (1986). Seed protein electrophoresis of diploids, tetraploids and tetraploid hybrids of *Capsicum* was initiated by Srivalli et al. (1999). Characterization of Nigerian varieties of *C. annuum* and *C. frutescens* by SDS PAGE of seed proteins was conducted by Odeigah et al. (1999). Identification of pepper cultivars by seed protein electrophoresis was also conducted by Lucchese et al. (1999). In chilli, work pertaining to the electrophoretic analysis of proteins and isozymes was restricted to species and variety differentiation, but comparative study of different lines of *C. annuum* was initiated in our laboratory. Seed proteins of 29 lines of *C. annuum* were analyzed by electrophoresis in the present study.

Materials and methods

Seeds used for extraction of proteins, were obtained from the Asian Vegetable Research and Development Center (AVRDC), Tainan, Taiwan, the Institute of Plant Genetic and Crop Plant Research, Gatersleben, Germany, the Kerala Agricultural University (KAU), Trichur, Kerala, India and the Indian Institute of Horticultural Research (IIHR), Bangalore, India. The dried seeds were homogenized with Tris HCl buffer

(0.05 M, pH 7.4) at 4 °C, for extraction of proteins. The homogenate was centrifuged at 10,000 g for 15 min at 4 °C and the supernatent used for electrophoresis. The protein percentage was estimated by Lowry's method (Lowry et al. 1951). The protocol for SDS polyacrylamide gel electrophoresis technique outlined by Hames (1994) was used to study the soluble protein pattern of the 29 lines of *C. annuum*. Electrophoresis was carried out using a Hoefer mini gel unit applying a constant current of 40 mA until the tracking dye, bromophenol blue reached the bottom. The gels were taken out and stained using Coomasie blue stain solution overnight and de stained using methanol (40%) and acetic acid (7%) solutions. The electrophorograms were prepared on the basis of protein mobility and the density expressed in Em values. The percentage of similarities between different lines was calculated as follows:

$$\% \text{ similarity} = \frac{\text{Number of pairs of similar bands}}{\text{Number of pairs of different bands} + \text{Number of similar bands}}$$

The morphological characters were recorded after evaluating the lines for three seasons. The colour value was estimated by the ASTA method (Hort and Fischer 1971) and the percentage of capsaicin by the ISO method (ISO DIS 7543-1).

Results

The electrophoretic profiles of the seed proteins from the representative genotypes were out lined in the form of an electrophorogram (Figure 1). Maximum no. of bands (21) were found in CAP 1086/35 (Acc. 27) & minimum no. (9) in PBC 385 (Acc. 9). The Em values ranged from 0.13 to 0.97. Each genotype was distinct from the other with respect to the Em values, but certain bands were shared by a few genotypes. The 29 accessions could be grouped into two big clusters (Figure 2), accessions 2 and 10–16 forming one cluster & the rest of the accessions forming the second cluster. Of these 10–16 were collected from AVRDC, while accession 2 was from IIHR. Details of the cluster groups are given under Table 2. A dendrogram drawn based on the percentage similarity, showed that there were 13 clusters (Table 1). Accessions 17–20 clustered together forming the first cluster. Of these 18, 19 and 20 were collected from KAU (Kerala) and were chilli types, whereas 17 was a paprika type collected from AVRDC. The second cluster was formed by accessions 21–24. Of these, 21 and 22 were chilli types whereas 23 and 24 were paprika types originating from USA. Accessions 25–28 formed the third cluster. Accessions 27 and 28 were chilli types from Institute of Plant Genetic and Crop Plant Research, Gatersleben, Germany, while 25 and 26 were from AVRDC. Accession 29 which formed the fourth cluster was a chilli type from Institute and Crop Plant Research, Gatersleben (IPK), Germany. Some paprika types, even though collected from IIHR, fell into different clusters 5, 6 and 7. The eighth cluster was formed by accession 9, which was a chilli type from Malaysia. Cluster nine was formed by accessions 12 and 13, which were collected from AVRDC. Accessions 15, 16 and 11, which were all chilli types from AVRDC, formed cluster 10. Cluster 11 was formed by accession 14 collected from AVRDC. Cluster 12 was formed by accession 10 from AVRDC and the 13th cluster by accession 2 collected from AVRDC. Average similarity was highest (80%) between accessions 17 and 19. Accession 17 (PBC 436) collected from AVRDC originated in Portugal (Table 1) and accession 19 (Round Ornamental) was collected from KAU. This suggests that the material collected from India possibly could have originated in Portugal.

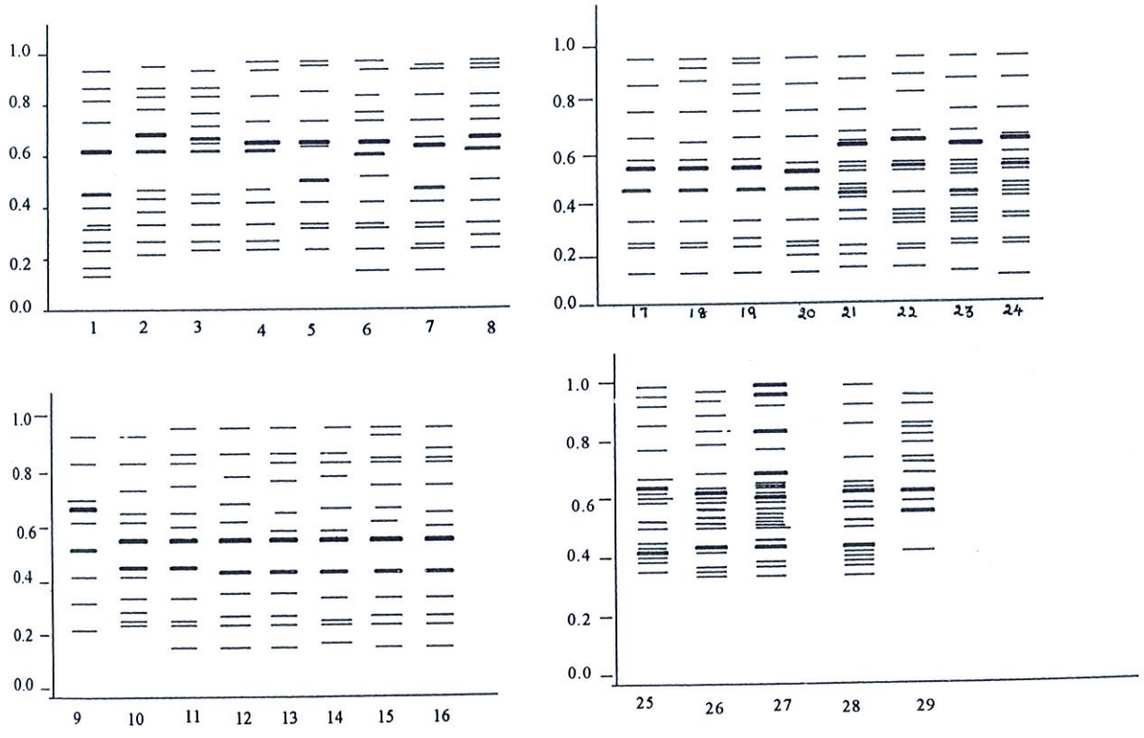

Figure 1. Electrophorograms showing the distribution of proteins in 29 accessions of *Capsicum annuum*.

The high seed protein lines clustered in different groups. Most of the paprika lines had high seed protein content and they also clustered together. Fruit length, fruit colour and percentage of capsaicin did not show correlations with the clustering of the accessions. But, in general, lines with common places of origin clustered together as also the high colour, low pungency paprika lines showed clustering together.

Discussion

The degree of homology in protein fractions appears to be a potential tool for measuring intra and interspecific relationships in chilli peppers (Panda et al. 1986). Gottlieb (1977) is of the opinion that electro-

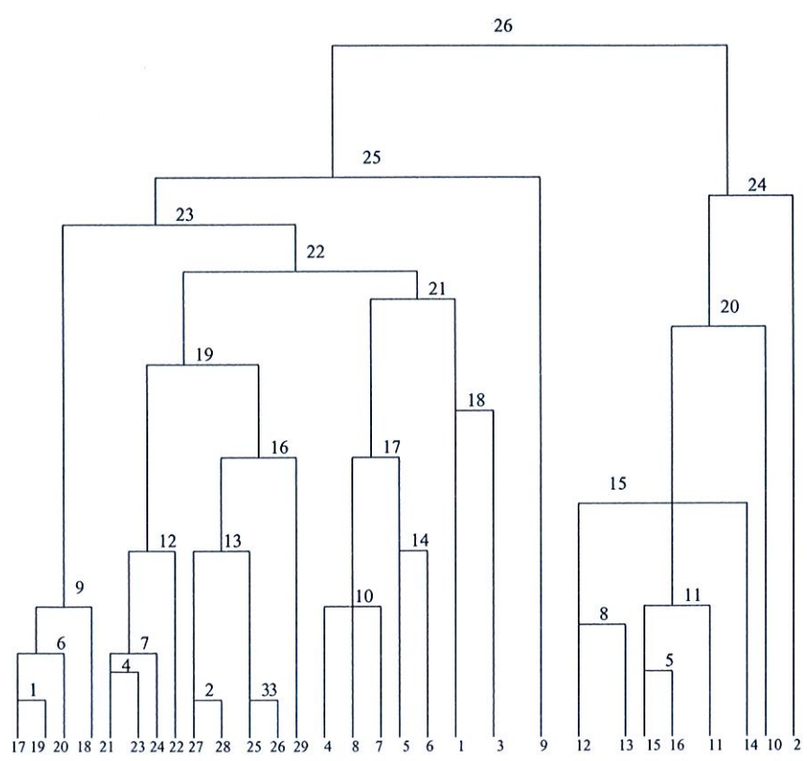

Figure 2. Cluster analysis dendrogram of 29 accessions of *Capsicum annuum* based on seed protein gel electrophoresis.

Table 1. Details of *Capsicum annuum* accessions used for screening.

Cluster	Accessions	Source/origin	Fruit type	Fruit shape	Fruit length	Fruit colour	% of capsaicin	% seed protein
1	17-PBC 436	AVRDC/Portugal	paprika	blocky	4.0	121	0.05	0.91
	18-Paprika type-1	KAU/India	paprika	triangular	3.1	116	0.26	2.8
	19-Round Ornamental	KAU/India	chilli	round	1.5	105	0.36	2.0
	20-Jwala	KAU/India	chilli	elongated	7.5	79	0.52	1.6
2	21-CA 219	KAU/India	chilli	elongated	6.1	117	0.53	3.4
	22-PBC 384	AVRDC/Malaysia	chilli	elongated	10.0	144	0.53	2.7
	23-PBC 828	AVRDC/USA	paprika	triangular	10.1	258	0.25	3.3
	24-PBC 999	AVRDC/USA	paprika	elongated	13.2	114	0.53	1.4
3	25-PBC 066	AVRDC/Malaysia	chilli	elongated	10.5	129	0.23	3.7
	26-PBC 554	AVRDC/Argentina	paprika	elongated	10.1	133	0.41	0.6
	27-CAP 1086/35	IPK, Gatersleben/Germany	chilli	triangular	2.5	89	0.60	1.1
	28-CAP 1063/35	- do -	chilli	elongated	5.3	188	0.30	1.4
4	29-CAP 1088/35	- do -	chilli	triangular	7.5	93	0.31	0.9
5	4-Kt-pl- 24	IARI regional station Katrain	paprika	elongated	10	148	0.29	3.0
	7-Kt-pl- 20	- do -	paprika	elongated	9.0	137	0.38	1.56
	8-Kt-pl- 8	- do -	paprika	triangular	7.1	102	0.28	5.4
6	5-Kt-pl- 19	- do -	paprika	triangular	6.5	225	0.21	3.42
	6-Kt-pl- 23	- do -	paprika	elongated	8.2	159	0.30	2.08
7	1-Kt-p1-22	- do -	paprika	elongated	9.0	107	0.29	3.5
	3-Kt-p1-18	- do -	paprika	elongated	7.5	134	0.14	2.2
8	9-PBC 385	AVRDC/Malaysia	chilli	elongated	10.5	172	0.47	2.2
9	12-PBC 1347	AVRDC/Malaysia	chilli	elongated	7.5	120	0.42	1.8
	13-PBC 971	AVRDC/Taiwan	paprika	elongated	9.1	119	0.24	3.3
10	11-PBC 743	AVRDC/Thailand	chilli	elongated	5.0	87	0.38	1.7
	15-PBC 375	AVRDC/Indonasia	chilli	elongated	10.0	101	0.37	1.6
	16-PBC 1350	AVRDC/Korea	chilli	elongated	6.0	105	0.26	2.6
11	14-PBC 535	AVRDC/Indonasia	chilli	elongated	12.0	86	0.22	0.6
12	10-PBC 473	AVRDC/Indonasia	chilli	elongated	9.9	112	0.47	1.7
13	2-Kt-p1-25	IARI/Katrain	paprika	triangular	9.0	223	0.16	3.4

Table 2. Cluster groups of the 29 accessions of *Capsicum annuum*.

Node	Groups	Average similarity	No. of objects in a fused group
1	Ac17 & Ac19	80	2
2	Ac27 & Ac28	76	2
3	Ac25 & Ac26	74	2
4	Ac21 & Ac23	72	2
5	Acc15 & Ac16	76	2
6	Ac17, Ac20 & Ac19	71	3
7	Ac21, Ac23 & Ac24	70	3
8	Ac12 & Ac13	70	2
9	Node6 & Ac18	65	4
10	Ac4,Ac8 & Ac7	65	3
11	Ac15,Ac11 & Ac16	75	4
12	Node7 & Ac22	66	4
13	Node2,Acc25 & Ac26	66	4
14	Ac5 & Ac6	54	2
15	Node8 & Ac14	57	3
16	Node13 & Ac29	54	5
17	Node10 & Node14	59	5
18	Acc1 & 3	46	2
19	Node12 & Node17	60	9
20	Node11,Node15 & Acc10	39	7
21	Node & Node19	53	7
22	Node20 & Node22	56	16
23	Node23 & Node9	55	13
24	Node21 & Acc2	34	8
25	Node24 & Acc9	25	21
26	Node26 & node25	29	29

phoretic difference rather than electrophoretic similarity is a valid criterion for assessment of genetic divergence. The study of similarity index gives an idea about the comparative gene homology between the different accessions of *C. annuum* and the average similarity suggests that there is considerable genetic diversity among the accessions.

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